

A High Throughput System for Profiling Respiratory Tract Microbiota

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INTRODUCTION

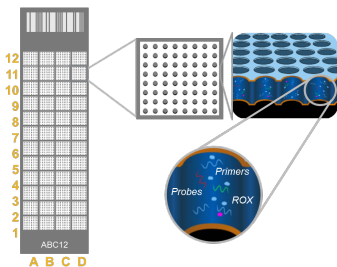
As one of the leading causes of death globally, respiratory infections could be caused by single or multiple types of viral, bacterial or fungal pathogens that present in the upper and lower respiratory tract. Panel-based testing using molecular methods to identify multiple pathogens simultaneously can contribute to better understanding of respiratory infections.

MATERIALS AND METHODS

To develop a comprehensive and flexible research panel, we chose TaqMan® OpenArray™ platform to identify common respiratory tract organisms via real-time PCR technology. Target organisms sequences were acquired from IMG and NCBI database. Divergent gene targets were chosen to design research assays with high specificity (ANI >96%) and wide strain coverage. For each target, multiple assays were designed in silico and then assay performance was evaluated using various controls including synthetic and natural genomic DNA and RNA, as well as human respiratory specimen. Assay specificity was evaluated with genomic RNA and DNA of standard reference viral and bacterial organisms from American Type Culture Collection (ATCC). To achieve higher sensitivity, optimized components and thermal cycling condition for PCR pre-amplification was determined by extensive Design of Experiments (DOE) studies.

RESULTS

Figure 1. OpenArray™ system for high throughput RTM profiling.



OpenArray™ technology utilizes a microscope slide-sized plate with 3,072 through-holes. Each plate contains 48 subarrays with 64 through-holes. Assays for different RTM targets are loaded in individual through-holes. 1-4 subarrays can form a RTM panel depending on customer specification of the number of targets and replicates. 12 – 48 samples may be processed simultaneously on one OpenArray plate for RTM studies.

Figure 2. RTM study workflow

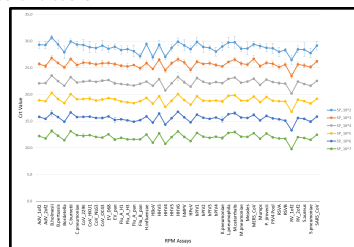


DNA and RNA from various types of samples of respiratory tract may be extracted with KingFisher™ Flex purification system, followed by reverse transcription and pre-amplification. The reaction is then loaded into OpenArray™ plates with AccuFill™ system. Real time PCR of RTM assays performed in QuantStudio™ 12K Flex instrument and results of positive or negative are analyzed with QuantStudio™ 12 Flex software.

Table 1. List of targeted organisms of Thermo Fisher Scientific OpenArray™ Respiratory Tract Microbiota (RTM) research panel

| Organism Name | Target Type | Assay Type |
|------------------------------|--------------------------|------------|
| Adenovirus pool 3_1 | Influenza A H3 | Viral |
| Adenovirus pool 3_2 | Influenza A pan-assay | |
| Bocavirus | Influenza B | |
| DNA: H1N1 (VZV) | Measles virus | |
| HRV4 (EBV) | MERS | |
| HRV5 (CMV) | Mumps virus | |
| HRV6 | RSVA specific | |
| Human Coronavirus 229E | RSV B specific | |
| Human Coronavirus HKU1 | SARS | |
| Human Coronavirus NL63 | Pneumocystis jirovecii | |
| Human Coronavirus OC43 | Bordetella | |
| Human coronavirus C229 | Bordetella holmesii | |
| Human enterovirus D68 strain | Bordetella pertussis | |
| Human enterovirus pan-assay | Chlamydia pneumoniae | |
| Human Metapneumovirus | Coccidioides immitis | |
| Human Parainfluenza virus 1 | Haemophilus influenzae | |
| Human Parainfluenza virus 2 | Klebsiella pneumoniae | |
| Human Parainfluenza virus 3 | Legionella pneumophila | |
| Human Parainfluenza virus 4 | Moraxella catarrhalis | |
| Human Parachovirus v2 | Mycoplasma pneumoniae | Bacterial |
| Human Rhinovirus A | Staphylococcus aureus | |
| Human Rhinovirus B/C | Streptococcus pneumoniae | |
| Influenza A H1 seasonal | | |

Figure 3. qPCR results of Thermo Fisher Scientific Respiratory Tract Microbiota (RTM) panel assays tested with synthetic assay targets (SP) at serial dilutions.



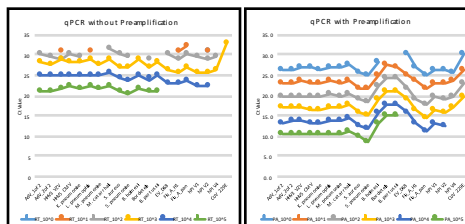
Plots show Ct values of selected assays at different input (copies/μL) of synthetic targets. The resulted Ct values show linear response to the copy number of targets, demonstrating high PCR efficiency, which confirms in silico design prediction.

Table 2. Specificity of OpenArray™ Respiratory Tract Microbiota (RTM) Panel

| Organism Name | Assay Name | Specificity |
|------------------------------|------------------------------|-------------|
| Adenovirus pool 3_1 | Adenovirus pool 3_1 | 100% |
| Adenovirus pool 3_2 | Adenovirus pool 3_2 | 100% |
| Bocavirus | Bocavirus | 100% |
| DNA: H1N1 (VZV) | H1N1 (VZV) | 100% |
| HRV4 (EBV) | HRV4 (EBV) | 100% |
| HRV5 (CMV) | HRV5 (CMV) | 100% |
| HRV6 | HRV6 | 100% |
| Human Coronavirus 229E | Human Coronavirus 229E | 100% |
| Human Coronavirus HKU1 | Human Coronavirus HKU1 | 100% |
| Human Coronavirus NL63 | Human Coronavirus NL63 | 100% |
| Human Coronavirus OC43 | Human Coronavirus OC43 | 100% |
| Human coronavirus C229 | Human coronavirus C229 | 100% |
| Human enterovirus D68 strain | Human enterovirus D68 strain | 100% |
| Human enterovirus pan-assay | Human enterovirus pan-assay | 100% |
| Human Metapneumovirus | Human Metapneumovirus | 100% |
| Human Parainfluenza virus 1 | Human Parainfluenza virus 1 | 100% |
| Human Parainfluenza virus 2 | Human Parainfluenza virus 2 | 100% |
| Human Parainfluenza virus 3 | Human Parainfluenza virus 3 | 100% |
| Human Parainfluenza virus 4 | Human Parainfluenza virus 4 | 100% |
| Human Parachovirus v2 | Human Parachovirus v2 | 100% |
| Human Rhinovirus A | Human Rhinovirus A | 100% |
| Human Rhinovirus B/C | Human Rhinovirus B/C | 100% |
| Influenza A H1 seasonal | Influenza A H1 seasonal | 100% |
| Influenza A H3 | Influenza A H3 | 100% |
| Influenza B | Influenza B | 100% |
| Measles virus | Measles virus | 100% |
| MERS | MERS | 100% |
| Mumps virus | Mumps virus | 100% |
| RSVA specific | RSVA specific | 100% |
| RSV B specific | RSV B specific | 100% |
| SARS | SARS | 100% |
| Pneumocystis jirovecii | Pneumocystis jirovecii | 100% |
| Bordetella | Bordetella | 100% |
| Bordetella holmesii | Bordetella holmesii | 100% |
| Bordetella pertussis | Bordetella pertussis | 100% |
| Chlamydia pneumoniae | Chlamydia pneumoniae | 100% |
| Coccidioides immitis | Coccidioides immitis | 100% |
| Haemophilus influenzae | Haemophilus influenzae | 100% |
| Klebsiella pneumoniae | Klebsiella pneumoniae | 100% |
| Legionella pneumophila | Legionella pneumophila | 100% |
| Moraxella catarrhalis | Moraxella catarrhalis | 100% |
| Mycoplasma pneumoniae | Mycoplasma pneumoniae | 100% |
| Staphylococcus aureus | Staphylococcus aureus | 100% |
| Streptococcus pneumoniae | Streptococcus pneumoniae | 100% |

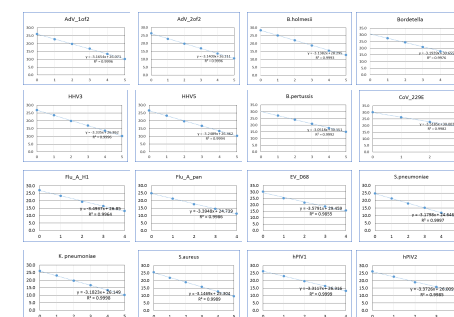
When tested with genomic RNA and DNA of standard reference viral and bacterial organisms from American Type Culture Collection (ATCC), the assays in RTM panel displayed high specificity. All organisms are detected by relevant assays.

Figure 4. Pre-amplification enhances assay sensitivity



Genomic materials from ATCC were pooled and titrated at serial dilutions for the tests. The left panel shows Ct values of pools when applied for real-time PCR test on OpenArray™ directly after reverse transcription. The right panel shows Ct values of corresponding pools underwent a pre-amplification process at which 14 cycles of polymerase chain reaction was carried out following reverse transcription.

Figure 5. Linearity range of RTM assays



Charts show Ct values (Y-axis) corresponding to different amount of initial input copies (X-axis, at power of 10 copies/μL) of ATCC viral and bacterial controls.

CONCLUSIONS

A novel research application has been developed for Respiratory Tract Microbiota (RTM) profiling. Assays in the panel demonstrate desirable performance in terms of sensitivity, specificity and linearity range. The application enables both customizable and high-throughput panels for respiratory infection research and provides a cost-effective tool for researchers to understand pathogenicity in respiratory tract infections.

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